

phosphoric acid or 10*N* sodium hydroxide. Dilute to volume with distilled water. Mix well.

(b) *Mobile phase.* Mix methanol:0.05*M* sodium phosphate buffer solution, pH 4.4 (5:95 v/v) and mix or ultrasonicate for no less than 2 minutes. Degas by passing through a 0.5-micron filter with vacuum. The mobile phase may be sparged with helium through a 2-micrometer metal filter for the duration of the analysis. Adjust the ratio of methanol to aqueous buffer as necessary to obtain satisfactory retention of the peaks.

(ii) *Working standard and sample solutions—(a) Preparation of working standard solution.* Accurately weigh and transfer into a 200-milliliter volumetric flask approximately 100 milligrams of amoxicillin working standard and approximately 50 milligrams of the clavulanate working standard. Dissolve and dilute to volume with distilled water. Use within 8 hours after preparation.

(b) *Preparation of sample solution.* Reconstitute the suspension as directed in the labeling. Immediately transfer an appropriate aliquot to a suitable volumetric flask to obtain an approximate amoxicillin concentration of 0.5 milligram per milliliter and dilute to volume with distilled water. Mix well for 10 minutes using a magnetic stirrer. Filter an aliquot through Whatman #42 or equivalent filter paper. Alternatively, a suitable membrane filter may be used. Samples should be prepared just prior to chromatographic injection. Inject the sample solution within 1 hour after the addition of water.

(iii) *System suitability requirements—*

(a) *Tailing factor.* The tailing factor (*T*) is satisfactory if it is not more than 1.5.

(b) *Efficiency of the column.* The efficiency of the column (*n*) is satisfactory if it is greater than 550 theoretical plates.

(c) *Resolution factor.* The resolution factor (*R*) between the clavulanic acid and amoxicillin peaks is satisfactory if it is not less than 3.5.

(d) *Coefficient of variation.* The coefficient of variation (*S_R* in percent) is satisfactory if it is not more than 2.0 percent.

If the system suitability requirements have been met, then proceed as described in § 436.351(b) of this chapter.

(iv) *Calculations.* Calculate the quantity of amoxicillin or clavulanic acid content in milligrams per milliliter of the oral suspension as follows:

$$\frac{\text{Milligrams of amoxicillin or clavulanic acid per milliliter}}{A_s} = \frac{A_u \times C \times V \times 0.5}{A_s}$$

where:

A_u=Response of the amoxicillin or clavulanic acid peaks in the sample chromatogram;

A_s=Response of the amoxicillin or clavulanic acid peaks in the standard chromatogram;

C=Concentration of the standard (milligrams per milliliter of amoxicillin X potency of amoxicillin standard or milligrams per milliliter of clavulanate X potency of clavulanate standard); and

V=Dilution volume in milliliters.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the suspension reconstituted as directed in the labeling.

[49 FR 39673, Oct. 10, 1984, as amended at 50 FR 19919, May 13, 1985; 55 FR 11582, Mar. 29, 1990]

§ 440.103f Amoxicillin trihydrate-clavulanate potassium chewable tablets.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity.* Amoxicillin trihydrate-clavulanate potassium chewable tablets are composed of amoxicillin trihydrate and clavulanate potassium with or without one or more suitable lubricants, diluents, flavorings, and binders. Each tablet contains amoxicillin trihydrate equivalent to either 125 or 250 milligrams of amoxicillin and clavulanate potassium equivalent to 31.25 or 62.5 milligrams of clavulanic acid. Its amoxicillin trihydrate content is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of amoxicillin that it is represented to contain. Its clavulanate potassium content is satisfactory if it contains not less than 90 percent and not more than 120 percent

of the number of milligrams of clavulanic acid that it is represented to contain. Its moisture content is not more than 6 percent. It passes the dissolution test if the quantity Q , of amoxicillin at 30 minutes, is 85 percent or greater. The amoxicillin trihydrate conforms to the standards prescribed by § 440.3(a)(1). The clavulanate potassium conforms to the standards prescribed by § 455.15(a)(1) of this chapter.

(2) *Labeling.* In addition to the labeling requirements prescribed by § 432.5 of this chapter, this drug shall be labeled “*amoxicillin-clavulanate potassium chewable tablets*”.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The amoxicillin trihydrate used in making the batch for potency, safety, moisture, pH, amoxicillin content, concordance, crystallinity, and identity.

(b) The clavulanate potassium used in making the batch for clavulanic acid content, moisture, pH, identity, and clavam-2-carboxylate content.

(c) The batch for amoxicillin content, clavulanic acid content, moisture, and dissolution rate.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(a) The amoxicillin trihydrate used in making the batch: 12 packages, each containing approximately 300 milligrams.

(b) The clavulanate potassium used in making the batch: 12 packages, each containing approximately 300 milligrams.

(c) The batch: A minimum of 100 tablets.

(b) *Tests and methods of assay—(1) Amoxicillin and clavulanic acid contents.* Proceed as directed in § 436.351 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength between 220 and 230 nanometers, and a column packed with microparticulate (3 to 10 micrometers in diameter) reversed phase packing material such as octadecyl hydrocarbon bonded silicas. Reagents, working standard and sample solutions, system suitability requirements, and cal-

culations for amoxicillin or clavulanic acid content are as follows:

(i) *Reagents—(a) 0.05M Sodium phosphate buffer solution, pH 4.4.* Transfer 7.8 grams of sodium monobasic phosphate to a 1-liter volumetric flask and dissolve in 900 milliliters of distilled water. Adjust the pH to 4.4 ± 0.1 with 18N phosphoric acid or 10N sodium hydroxide. Dilute to volume with distilled water. Mix well.

(b) *Mobile phase.* Mix methanol: 0.05M sodium phosphate buffer solution, pH 4.4 (5:95 v/v) and ultrasonicate for no less than 2 minutes. Degas by passing through a 0.5-micron filter with vacuum. The mobile phase may be sparged with the helium through a 2-micrometer metal filter for the duration of the analysis. Adjust the ratio of methanol to aqueous buffer as necessary to obtain satisfactory retention of the peaks.

(ii) *Working standard and sample solutions—(a) Preparation of working standard solution.* Dissolve and dilute accurately weighed portions each of the amoxicillin trihydrate working standard and the clavulanate lithium working standard with water to obtain a solution containing 0.5 milligram of amoxicillin and 0.25 milligram of clavulanic acid per milliliter. Use within 1 hour after preparation or within 4 hours if stored under refrigeration.

(b) *Preparation of sample solution.* To obtain a concentration of 0.5 milligram of amoxicillin per milliliter, dissolve a representative number of tablets in water with the aid of a magnetic stirrer or ultrasonication. Filter an aliquot through Whatman #42 filter paper or equivalent, discard the first 10 milliliters of filtrate, and use the remaining portion as the sample solution. Alternatively, a suitable membrane filter may be used. Prepare samples not more than 1 hour before the chromatographic injection.

(iii) *System suitability requirements—(a) Tailing factor.* The tailing factor (T) is satisfactory if it is not more than 1.5.

(b) *Efficiency of the column.* The efficiency of the column (n) is satisfactory if it is greater than 1,000 theoretical plates in a 30-centimeter column for each active component.

(c) *Resolution.* The resolution (R) between the clavulanic acid and amoxicillin peaks is satisfactory if it is not less than 3.5.

(d) *Coefficient of variation.* The coefficient of variation (S_R in percent) of five replicate injections is satisfactory if it is not more than 2.0 percent.

If the system suitability requirements have been met, then proceed as described in § 436.351(b) of this chapter.

(iv) *Calculations.* Calculate the milligrams of amoxicillin or clavulanic acid content per tablet as follows:

$$\frac{\text{Milligrams of amoxicillin or clavulanic acid per tablet}}{A_s \times N} = \frac{A_u \times C_s \times V}{A_s \times N}$$

where

A_u =Response of the amoxicillin or clavulanic acid peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s =Response of the amoxicillin or clavulanic acid peak in the chromatogram of the amoxicillin or clavulanic acid working standard;

C_s =Concentration of standards in milligrams of amoxicillin or clavulanic acid per milliliter of the standard solution;

V =Volume of sample solution (milliliters); and

N =Number of tablets taken for assay.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *Dissolution.* Proceed as directed in § 436.215 of this chapter. Dissolution rate is determined by dissolution of the amoxicillin component using the high-performance liquid chromatographic assay described in this section.

[50 FR 42933, Oct. 25, 1985; 50 FR 47367, Nov. 17, 1985, as amended at 55 FR 11582, Mar. 29, 1990]

§ 440.105 Ampicillin oral dosage forms.

§ 440.105a Ampicillin tablets.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity.* Ampicillin tablets are composed of ampicillin with one or more suitable and harmless diluents and lubricants. Each tablet contains 250 or 500 milligrams of ampicillin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of ampicillin that it is represented to

contain. Its loss on drying is not more than 4 percent. The tablets disintegrate within 15 minutes. The ampicillin used conforms to the standards prescribed by § 440.5(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The ampicillin used in making the batch for potency, loss on drying, pH, ampicillin content, concordance, crystallinity, and identity.

(b) The batch for potency, loss on drying, and disintegration time.

(ii) Samples required:

(a) The ampicillin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(b) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay—(1) Potency.* Use either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of tablets into a high-speed glass blender jar with sufficient 0.1M potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot and further dilute with solution 3 to the reference concentration of 0.1 microgram of ampicillin per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, except in paragraph (d) of that section, add 3 drops of 1.2N hydrochloric acid to both the sample and working standard solutions after the addition of 0.01N iodine solution. Prepare the sample as follows: Place a representative number of tablets in a high-speed glass blender jar and add sufficient distilled water to give a convenient concentration. Blend for 3 to 5 minutes. Further dilute an aliquot with distilled water to the prescribed concentration.

(2) *Loss on drying.* Proceed as directed in § 436.200(a) of this chapter.